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A pharmaceutical delivery system for vitamin C and vitamin E and use of a combination of vitamin C and E for preventing or treating conditions involving oxidative stress

This invention relates to a pharmaceutical delivery system for obtaining a controlled ratio of antioxidants or antioxidant drugs in blood plasma.

It is the object of the present invention to prevent or treat arteriosclerosis or other diseases or conditions where reactive oxygen species are involved.

The present invention proposes a pharmaceutical delivery system for oral delivery of the antioxidants vitamin C and vitamin E to obtain high concentrations thereof and a controlled ratio between the vitamins in blood plasma in humans and animals.

Free radical chemistry appears in the cells of all mammalian bodies. Free radicals relating to oxygen are of particular importance because of the use of oxygen to generate energy in the body. In the cellular processes oxygen is reduced to water through the addition of 4 electrons, a process that is tightly controlled (Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiology Review 1979; 59:527-605). Intermediary products (reactive oxygen species, ROS) are produced, i.e. superoxide anions - hydrogen peroxide - hydroxyl radicals. The ROS are highly reactive and modify important cellular macromolecules (Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause or consequence. Lancet 1994; 344:721-724.), and thereby initiate or accelerate disease processes.

The formation of ROS can occur as part of many cellular processes including mitochondrial respiration, immune cell responses, cell injury, heat, radiation of many origins, from metabolism of drugs and other chemicals. ROS are thought to be involved in almost all disease processes and the ageing process.

For example, modification can occur to lipids in the LDL (light density lipoprotein) particle in the blood (Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radical Research Communications 1989; 6:67-75. and ; Esterbauer H, Gebicki J, Puhl H, Günther J. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radicals in Biology and Medicine 1992; 13:341-390). This modification leads to increased formation of fatty streaks in the arterial wall and subsequent formation of arterisclerotic plaques (Esterbauer et al. supra and Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-lipoprotein that increase its atherogenecity. N Engl J Med 1989; 320(14):915-924) which can compromise blood supply to organs, causing manifest disease, e.g. coronary heart attack.

The body and its cells have several mechanisms to control the effects of ROS. The general term of such mechanisms is antioxidants. Antioxidants include enzymes, substances produced in the body and substances that are only found in food. Examples of the latter are antioxidant vitamins (E,C,A) and similar substances (flavonoids, lucupene, beta-carotene). The substances have different properties, some being water-soluble others being fat-soluble (Halliwell supra).

During the last decades, evidence has gathered linking both high intake of food rich in antioxidants, and intake of supplements containing antioxidant vitamins to reduce incidence of cancer and arteriosclerosis (Hennekens CH, Gaziano JM, Manson JE, Buring JE. The antioxidant vitamin-cardiovascular disease hypothesis is still promising, but remains unproved: the need for randomised trials. Am J Clin Nutr 1995; 62:1377S-1380S).

A particularly important part of the lipid phase is the LDL particle (low density lipoproteins). These particles are produced in the liver and are responsible for transport of lipids, particularly cholesterol. These particles are taken up by cells by a protein moiety APO-B100, an uptake which is feed-back inhibited. If LDL is oxidised, it cannot be taken up, but is then devoured by monocyte derived macrophages with no feed-back inhibition. Macrophages can transform into foam cells when large amounts of LDL are taken up. The foam cells deposit in the arterial wall and contribute to the development of artheriosclerotic plaques (Steinberg et al. supra).

Water-soluble antioxidants are taken up quickly, but are also eliminated quickly from the body by urinary excretion (Levine M, Dhariwal KR, Welch RW, Wang Y, Park JB. Determination of optimal vitamin C requirements in humans. Am J Clin Nutr 1995; 62:1347S-1356S). Fat-soluble antioxidants are taken up more slowly and eliminated slowly from the body (Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 1990; 10:357-382). This means that the concentration ratio of e.g. a water-soluble and a fat-soluble antioxidant vitamin will vary after intake.

Water-soluble and fat-soluble antioxidants are found in the water phase and in the lipid phase of the body, respectively. In the transition phase between the lipid and water phases there is co-operation between the water and the fat-soluble antioxidants. An example of this is the interaction between vitamin C and vitamin E in the transition between the LDL particle and the water phase of the blood (Kagan VE, Serbinova EA, Forte T, Scita G, Packer L. Recycling of vitamin E in human low density lipoproteins. J Lipid Res 1992; 33:385-397 and; Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and betacarotene. Am J Clin Nutr 1995; 62:1322s-1326s). Vitamin E is the most important antioxidant in the LDL particle.

When vitamin E is oxidized in the LDL particle, a tocopheryl radical is generated. This radical can elicit lipid peroxidation or protein oxidation and can thus result in the oxidation of the LDL particle with the consequences described above (Kagan et al supra). Vitamin C, ascorbic acid (AA), can prevent this process by interacting with the tocopheryl radical. This results in reduction of the tocopheryl radical to tocopherol and the formation of oxidised vitamin C, dehydro-ascorbic acid, DHAA (D. Horring, S. Afr. Med. J. 60, 818-823, 1981). DHAA is taken up by the liver and reduced to vitamin C (Washko PW, Welch RW, Dhariwal KR, Wang Y, Levine M. Ascorbic acid and dehydroascorbic acid analyses in biological samples. Anal Biochem 1992; 204:1-14).

The present invention is based on the assumption that a certain ratio between vitamin C (ascorbic acid) and vitamin E (α-tocopherol) is necessary for optimum protection of LDL particles.

The present invention solves the problem of providing high concentrations of vitamin C and E in the preferred ratio by using a pharmaceutical delivery system for oral delivery of vitamin C and vitamin E to obtain high concentrations thereof in a controlled ratio in blood plasma in humans or animals by a delivery system with slow release of vitamin C and plain release of vitamin E.

It is preferably a two layer tablet comprising vitamin C in one layer and vitamin E in the other layer, but the delivery system of the invention can be any system providing a high concentration of vitamin C and vitamin E at the same time in the blood.

US 5897879 discloses a sustained-release pharmaceutical delivery system for the administration of an antioxidant drug to a patient in need of such drug, wherein the said delivery system comprises the said drug in combination with a matrix, the said matrix comprising a polymer selected from the group consisting of a polymer which does not interact with the said drug and a mixture of such polymers, and the said polymeric matrix is present in amounts from about 20% (w/w) to about 80% (w/w). The drug can inter alia be vitamin E, vitamin C or a combination thereof. In the case of combination both drug components have a sustained-release form, and they are released together. This known system does not give effective high, constant concentrations of vitamin C and E.

WO 97/00672 discloses an effervescent composition comprising at least one active ingredient selected from the group consisting of a nutritional supplement, a dietary supplement and combinations thereof in amounts sufficient to provide a dosage form of the said active ingredient as few as once in a 24-hour period, the said

active ingredients being both a free form component and a microencapsulated component which has sustained-release properties, and an effective amount of an effervescent agent. The active agent is selected from the group consisting of carnitine, calcium, magnesium, ascorbic acid, vitamin E and combinations thereof. The active agents are micro-encapsulated together. This known composition provides immediate and sustained release of both vitamin C and vitamin E. The vitamins are released together from the same delivery principle(s), and they do not provide a high, constant vitamin concentration, in the preferred ratio in the blood plasma.

EP 176772 discloses a process for increasing the delayed-release activity of vitamin C and vitamin E by incorporating both vitamins in a neutral oil and encapsulating the oil. Both vitamins are present in the same delivery principal.

Surprisingly, it has now been found that the delivery system of the invention providing slow release of vitamin C and plain release of vitamin E can give a high, constant concentration of the vitamins in the blood of a human or an animal in need of the vitamins. This can be used for patients having vitamin C and/or E deficiency, but especially for preventing or treating conditions or diseases involving oxidative stress, such as arteriosclerosis, cancer, cataract, diabetes I and II, and ageing. Many human studies have been performed in this area.

A large controlled trial over 5+ years showed no effect of a 50-mg tocopherol dose (Heinonen OP, Albanes D. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994; 330:1029-1035).

A controlled release formulation of vitamin C and E failed to give the expected increase in vitamin E plasma concentrations whereas it produced a more constant and thus favourable vitamin C concentration (Nyyssonen K, Poulsen HE, Hayn M, Agerbo P, Porkkala-Sarataho E, Kaikkonen J et al. Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation. European Journal of Clinical Nutrition 1997; 51(3):154-163.

The system of the invention is characterised by slow release of vitamin C and plain release of vitamin E.

The system can be a pharmaceutical delivery system comprising a tablet comprising two or more different delivery principles, wherein (A) one delivery principle comprises (i) vitamin C (ii) a pharmaceutically acceptable excipient for controlling the slow release of vitamin C, (iii) other pharmaceutically acceptable excipients (B), another delivery principle comprises (i) vitamin E (ii) pharmaceutically acceptable excipients.

The system can of course be any known delivery system providing slow release of one ingredient and plain release of another ingredient. The following discusses the inventors' thoughts behind formulation of the antioxidants, and especially the antioxidants vitamin C and vitamin E.

Description of release properties

It is known that absorption of vitamin C improves when the active ingredients are released from the tablet, making it accessible for absorption over a period of 7-9 hours (Bhagavan et al., Correlation Between the

Disintegration Time and the Bioavailability of Vitamin C-tablets, Pharmaceutical Research, Vol. 10, No.2, 1993). This type of formulation is called a sustained release formulation, extended release formulation, prolonged release formulation, slow release formulation or modified release formulation.

Sustained release formulations

Sustained release formulations are known to give lower peak values than other administration forms, but keep the desired plasma level for a longer time (H.Gjelstrup Kristensen, N. Møller, Almen Farmaci I, Dansk Farmaceutforenings forlag, 1980: p.93 - 97). With repeated dosage of the sustained release formulation, a much more constant plasma level can be obtained compared to conventional tablets.

Sustained release formulation can be achieved by different techniques, such as matrix tablets, erosion tablets, lattice tablets, or by coating of the tablet or the active ingredient.

The matrix principle, which is used in this tablet, is achieved by mixing the active ingredient with hydrocolloid macromolecular excipients in large amounts, typically more than 25%. When ingested, the tablet forms a highly viscous gelatinous mass at the surface maintaining the shape of the tablet. The active component is slowly released from the surface of the gelatinous mass, at a rate which is controlled by its diffusion through the gel-barrier.

The following macromolecular excipients can be used for creating this gel: Methylcellulose, Hydroxypropyl methylcellose, Carboxymethyl starch or other modified

cellulosic substances, hydrophilic gums such as pectinates or alginates.

Erosion tablets differ from the matrix tablet in that the excipients used are lipids, which will not dissolve or gel in the stomach, but slowly be eroded, thus releasing the active ingredient. The following lipids are frequently used for this purpose: stearic acid, glycerol monostearate, stearyl alcohol, cetyl alcohol, and hydrogenated fats.

Lattice tablets differ from the former types in that the excipient chosen is insoluble in the stomach. The tablet will therefore not disintegrate, and the active ingredient is released by diffusion, leaving the lattice unchanged. As excipients for lattice tablets, polyvinyl acetate, polyvinyl chloride or polyethylene is used.

The sustained release effect can also be achieved either by coating the tablet or by coating the active particles or pellets made herefrom (micro-encapsulation). The coating must be made of an insoluble polymer, which the active ingredient has to pass by diffusion. As polymers for film coating, ethyl cellulose, polymetacrylates or lipids are used.

It is not possible to apply coating to whole tablets in such cases where different release profiles of different parts of the tablet are desired.

Antioxidant formulation

According to one aspect, the invention comprises a bi-layer tablet formulated with vitamin C and vitamin E for prevention/treatment of oxidative stress related indications.

Vitamin C has been formulated for sustained release by a matrix principle in the first layer of the tablet. Vitamin E, however, is released immediately by disintegration of the second layer.

Vitamin C layer

In this formulation the matrix technique with hydroxypropyl methylcellulose as gel forming excipient has been chosen. This is based on good experience in making sustained release vitamin C tablets by this technique, and because the release profile from experience is relatively low sensitive to differences in production parameters.

Figure 1 shows the release pattern measured on different batches.

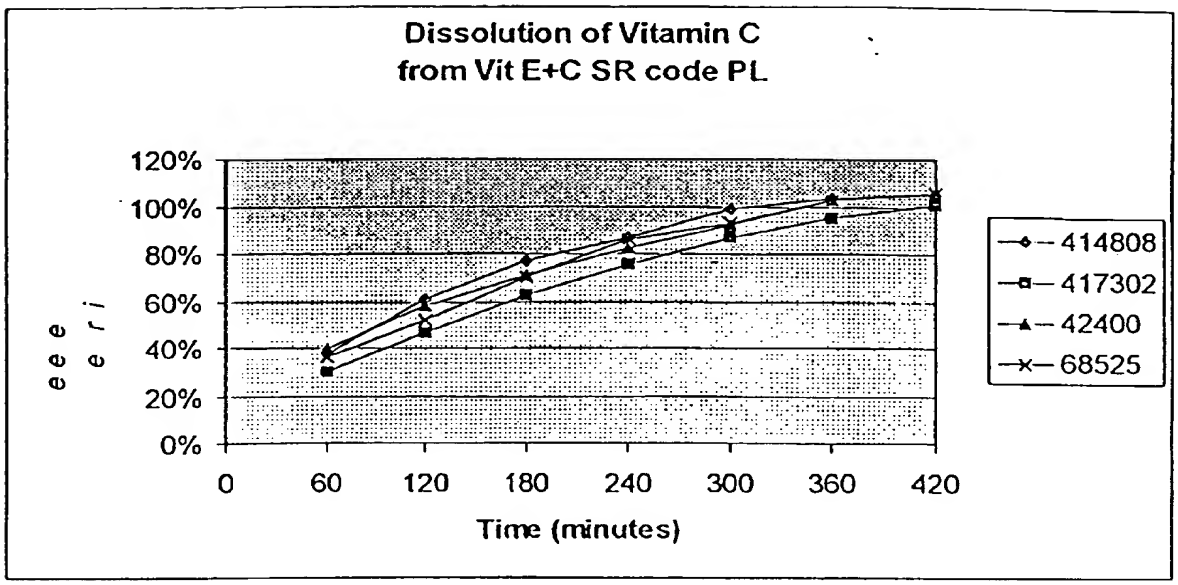


Figure 1

The graph demonstrates that vitamin C is released over 7 hours, and that the production process is reproducible in relation to release of vitamin C.

Vitamin E layer

This layer is formulated as a conventional tablet meeting the normal requirements for disintegration.

It has been shown that if vitamin E (α-tocopheryl acetate) is mixed into the sustained release vitamin C matrix, vitamin E is not absorbed very well (Salonen et al., Am. J. Nutr., A randomised, single blind, placebo-controlled trial of the effects of 200 mg α-tocopherol on the oxidation resistance of artherogenic lipoproteins, 1998;68:1034-41). On the other hand, vitamin E given in a conventional tablet with similar formulation is well absorbed. Vitamin E is therefore to be administered in a form like a conventional tablet, which has to disintegrate within 30 minutes, making vitamin E accessible for absorption.

Choice of active and inactive ingredients

In the formulation, ascorbic acid and d-α-tocopheryl acatate have been used as sources for the active components.

It is expected that any other form of vitamin C (e.g. sodium ascorbate, calcium ascorbate and ascorbyl palmitate), and any other form of vitamin E (e.g. d,l-α-tocopheryl acatate and d-α-tocopheryl succinate) will be absorbed to the same degree if administered with the described release characteristics.

Likewise, it is expected that making the vitamin C sustained release by other hydrocolloids (as those described earlier) or any other technique (as those described earlier) will have the same effect on the absorption of the product.

The system of the invention can provide a ratio between vitamin C and vitamin E in the blood plasma ranging from 1:10 and 10:1, preferably 1:5 and 5:1, and especially 1:1 and 3:1. The amount of vitamin C is preferably higher than that of vitamin E. The most preferred ratio is 2.2:1

The concentration of vitamin C in human blood should be above 10 µmol/litre, preferably above 20 µmol/litre, and the concentration of vitamin E above 15 µmol/litre preferably above 50 µmol/litre. Using the system of the invention it has been possible to reach a concentration of vitamin C of about 180 µmol/litre, and a concentration of vitamin E of about 180 µmol/litre.

This can be achieved by administration of a daily dose of 1-4 tablets, each containing 250 mg of vitamin C and 91 mg of vitamin E, preferably 2 tablets a day. The same amounts of vitamin C and vitamin E can also be achieved by other tablets or delivery principles.

Vitamin C is ascorbic acid, a derivative or a salt thereof, for example sodium ascorbate, calcium ascorbate or ascorbyl palmitate.

Vitamin E is any natural or synthetic vitamin chosen from the group comprising d-α-tocopheryl acetate, d-α-tocopheryl acid succinate, d-α-tocopherol, d-β-tocopherol, d-γ-tocopherol, d-δ-tocopherol, d-α-tocotrienol, d-β-tocotrienol, d-γ-tocotrienol, d-δ-tocotrienol, dl-α-tocopherol, dl-α-tocopheryl acetate, dl-α-tocopheryl calcium succinate, dl-α-tocopheryl nicotinate, dl-α-tocopheryl linoleate/oleate and all other possible stereo isomeric forms of the above compounds.

Pharmaceutical results

Example

A tablet according to the invention was prepared as follows:

Manufacturing formula

Batch size: 1.050 million tablets

I	D-α-Tocoferol Acetate Concentrate (Powder form)	approx. [1]	102.000	kg
	Silica, Colloidal Anhydrous		10.500	kg
	Cellulose, Microcrystalline	approx. [2]	59.700	kg
II	D-Alpha-Tocoferol Acetate Concentrate (Powder form)	approx. [1]	102.000	kg
	Silica, Colloidal Anhydrous		10.500	kg
	Cellulose, Microcrystalline	approx. [2]	59.700	kg
III	Magnesium Stearate		2.100	kg
IV	Hypromellose 100000		131.900	kg
	Ascorbic Acid 97%		270.620	kg
V	Magnesium Stearate		0.680	kg
VI	Water, Purified		75.000	kg
	Ethanol 96%		12.000	kg
	Titanium Dioxide		0.730	kg
	Riboflavine		0.510	kg
	Hypromellose 3		2.000	kg
	Hypromellose 15		4.000	kg
	Glycerol 85%		1.200	kg
VII	Talc		0.040	kg

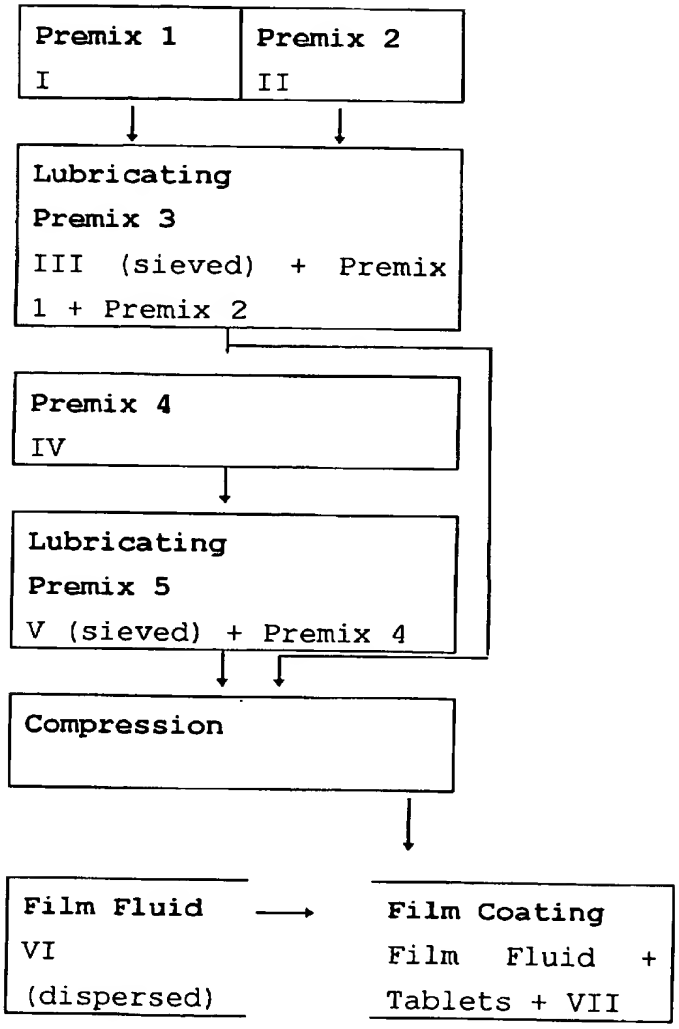
[1] Amount adjusted according to the result of the assay of the raw material.

[2] Amount adjusted in order to balance the mass to 172.200 kg each for I and II.

Manufacturing process

Flow-chart

Process operation



Description

D-α-tocoferol acetate concentrate (powder form), silica, colloidal anhydrous, and microcrystalline cellulose are mixed for 15 minutes (I).

The above procedure is repeated (II).

Sieved magnesium stearate, Premix 1, and Premix 2 are mixed. (III).

Hypromellose 100000, and ascorbic acid 97% are mixed for 30 minutes (IV).

Sieved Magnesium Stearate is added and the blend is mixed (V).

Premix 3 and Premix 5 are compressed into 2-layer tablets with 12.0 mm punches. Premix 3 is compressed as the first layer.

Titanium dioxide, riboflavine, hypromellose 3, hypromellose 15, and glycerol 85% are dispersed in purified water and ethanol 96%. (VI).

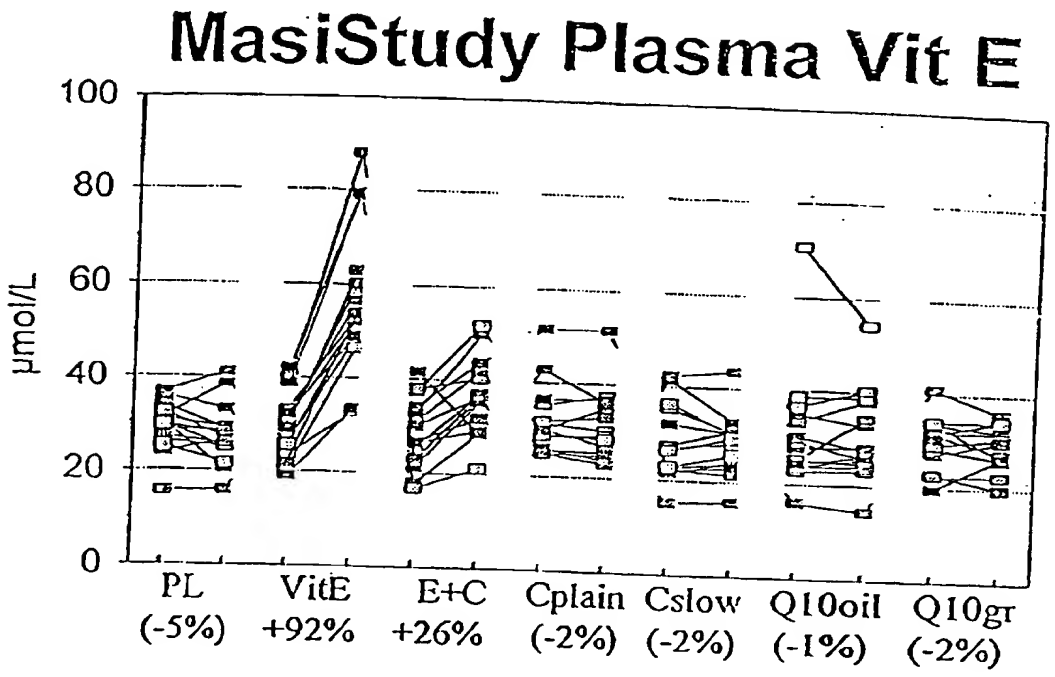
The film fluid is sprayed upon the tablets in sub-batches of approx. 236 kg.

Talc is sprinkled upon the tablets (VII).

This tablet was used in a controlled trial on humans.

In an unpublished study the inventors have previously found that slow release vitamin E combined with vitamin C, in a similar test, gives poor bioavailability of vitamin E compared to a conventional vitamin E formulation.

"MasiStudy Plasma Vit E"



Summary of plasma vitamin E measurement in the MASIstudy (Eur J Clin Nutr, 1997; 51(3); 154-163) at baseline and after two months supplementation.

The unpublished data from MASIS include plasma concentration in 7 groups (PL = placebo, VitE = normal formulation vitE, E+C = Slow release vitE combined with vitC, Cplain = plain formulation of vitC, Cslow = slow release formulation of vitC, Q_{10}oil = Coenzyme Q_{10} formulated in oil, Q_{10}gr = Coenzyme Q_{10} in granulate formulation). Vitamin amounts: E = 2 x 45,5 mg; C = 2x 250 mg; Q_{10} = 3 x 30 mg.

The results show that the bioavailability of vitamin E, slow release formulation, was poor compared to the conventional vitamin E formulation. On the basis of these findings, a formulation was developed as one tablet where vitamin E matrix gave optimum release, as in the conventional formulation, and vitamin C was slow release, since this provided more even plasma vitamin C concentrations over time.

Such favourable effect of a targeted increase in both vitamin C and vitamin E plasma concentrations lead to that the inventors conducted a controlled trial with a formulation including both slow release vitamin C and conventional release vitamin E (250 mg AA and 91 mg α-tocopherol). 520 men and women, smokers and non-smokers, were randomised to vitamin C slow release, vitamin E, a formulation with vitamin E and slow release vitamin C, or placebo. The combined formulation gave a 72 - 89 percent increase in plasma vitamin E and a 60- 72 percent increase in plasma vitamin C in plasma (morning values). In smoking men, the group with considerable oxidative stress, the progression rate of the thickness of the

arteria intima was reduced from 0.020 mm/yr on placebo to 0.011 mm/yr after the combined formulation $p<0.05$. This corresponds to almost halving the progression of the process that can later manifest itself as arteriosclerosis. The thickness of the carotid intima was measured with ultrasound, this measurement has shown to be predictive of coronary heart disease and may be predictive of other arteriosclerotic manifestations as well. The results are discussed in an article by one of the inventors, J.T. Salonen et al. "The Effect of Vitamin E and Vitamin C on 3-year Progression of Carotid Atherosclerosis: the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study" (in print and quoted in the following):

The effect of vitamin E and vitamin C on 3-year progression of carotid atherosclerosis: the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study

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Summary

Background Dietary and self-selected supplementation of vitamin E has been associated with a reduced incidence of coronary events, but the evidence from randomised clinical trials is controversial. We studied the efficacy of vitamin E and C supplementation on the progression of carotid atherosclerosis, hypothesising an enhanced preventive effect in men and in smokers and synergism between vitamins.

Methods In a double-masked 2x2 factorial trial, 520 smoking and non-smoking men and postmenopausal women aged 45-69 years with serum cholesterol ³5.0 mmol/L were randomised in these four strata to receive either 182 mg of d-a-tocopheryl acetate, 500 mg of slow-release vitamin C daily, both or placebo for three years. Atherosclerotic progression was defined as linear regression slope of the mean ultrasonographically assessed common carotid intima-media thickness (IMT) over time.

Findings The average increase of the mean IMT was 0.020 mm/year among men who were randomised to only placebo and 0.018 mm/year in vitamin E, 0.017 mm/year in vitamin C and 0.011 mm/year in the double vitamin group (p=0.009 for E+C vs other men). The respective means in women were 0.016, 0.015, 0.017 and 0.016 mm/year. The proportion of men with progression was reduced by 74% (95% CI 36-89%, p=0.003) by

supplementation with both vitamins, as compared with placebo. This protective effect was greatest in smoking men and absent in women.

Interpretation In conclusion, our study shows that a combined supplementation with reasonable doses of both vitamin E and vitamin C for at least three years can retard the progression of common carotid atherosclerosis substantially in regularly smoking hypercholesterolemic men. This may imply benefits with regard to other atherosclerosis-based events.

Introduction

Evidence from both basic research and epidemiology indicates that enhanced lipid peroxidation is associated

with accelerated atherogenesis,[1-5] whereas that from randomised clinical trials is very limited and controversial.[5-12] While epidemiologic studies suggest that lipid peroxidation might have its greatest relevance in the early phases of atherosclerotic lesion development [1,2,4,5,8] and that vitamin E may have a protective effect, if any, in clinically healthy persons, [13-18] there are no previous studies testing the hypothetical preventive effect of vitamin E on atherosclerotic progression in clinically healthy subjects.

Vitamin E and vitamin C are considered two of the most important dietary antioxidants.[5,7,17-19] Vitamin E may also have other antiatherogenic properties.[20] When vitamin E works as an antioxidant it is oxidised to harmful a-tocopheroxyl radical, which needs to get reduced back to a-tocopherol. Vitamin C can regenerate a-tocopheroxyl radical to a-tocopherol.[21] Theoretically, supplementing high-risk individuals with high doses of vitamin E alone could even promote rather than reduce lipid peroxidation.[22] Also, in our prospective population study, vitamin C deficiency was associated with increased risk of coronary events.[23] For these reasons we designed a randomised clinical trial in which both vitamin E and vitamin C were supplemented in a factorial design.

The main purpose of the ASAP (Antioxidant Supplementation in Atherosclerosis Prevention) study was to test the effect of reasonable supplemented doses of vitamin E and vitamin C and their combination on the progression of common carotid atherosclerosis in middle-aged high-risk men and women in three years. As men and cigarette smokers are at enhanced oxidative stress and lipid peroxidation,[1,7] a greater atherosclerotic progression retarding effect was hypothesised a priori in men and in smokers than in women and in non-smokers. Because of the

synergism between vitamin E and vitamin C in the human body, the greatest protective effect was hypothesised by the combined supplementation.

Methods

Study design, inclusion and exclusion criteria and supplements

The ASAP study was designed to test the main study hypothesis that the supplementation of 45-69 -year old smoking and non-smoking men and postmenopausal women with either 200 mg of d-a-tocopheryl acetate or 500 mg of vitamin C daily or both will retard the progression of common carotid atherosclerosis, the elevation of blood pressure and the progression of cataracts. This report concerns the effect on atherosclerosis. ASAP is a clinical placebo-controlled double-masked 2x2 factorial trial. All subjects had hypercholesterolemia, defined as serum cholesterol of ³5.0 mmol/L at screening.

Subjects were not entered into the trial if they had: premenopause or regular oral estrogen substitution therapy in women, regular intake of antioxidants, acetosalicylic acid or any other drug with antioxidative properties, severe obesity (BMI > 32 kg/m^2), type 1 diabetes, cataracts extracted bilaterally making opacity assessment impossible, uncontrolled hypertension (sitting diastolic BP >105 mmHg), any condition limiting mobility, making study visits impossible, severe disease shortening life expectancy, or other disease or condition worsening the adherence to the measurements or treatment.

The study consisted of 8-week dietary counseling and placebo lead-in phase and a 3-year double-masked phase, for which the subjects were randomly allocated to either

(1) 100 mg of d-a-tocopheryl acetate twice daily (272 IU of vitamin E a day), (2) 250 mg slow-release ascorbic acid twice daily, (3) both d-a-tocopheryl acetate and ascorbic acid in a single tablet, or (4) placebo only. After the double-blind 3-year period, the study is continuing for another three years as an open study. The doses were chosen on the basis of pilot and kinetic studies.[24,25] The subjects were randomised separately in four strata of approximately equal size: (1) smoking (≥5 cigarettes/day) men, (2) nonsmoking men, (3) smoking postmenopausal women, and (4) nonsmoking postmenopausal women. All subjects gave a written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

The subjects came to baseline visits and were randomised between October 1994 and October 1995. Follow-up visits were 6, 12, 18, 24, 30 and 36 months later. Supplements were given, returned tablets were counted and ultrasonographic assessment of common carotid artery (CCA) intima-media thickness (IMT)[14,26] was carried out at all these seven visits.

Power analysis

Based on our previous studies,[14] we assumed that the placebo group will have an average slope of CCA-IMT increase of 0.03 mm/year. The goal for the sample size was set at 500 randomised subjects (expectedly 125 in each stratum), which was expected to result in 429 participants at the end of the 3-year period at an annual drop-out rate of 5%. A 25% treatment effect was expected, detectable at $a=0.05$ with power of >0.80 within gender for vitamin E plus C group compared with other treatment groups.

Study participants

After screening of volunteers in phone, 946 eligible persons were invited to screening, 803 were examined and 660 persons were entered into a 8-week run-in phase. Of these, 520 subjects (256 men and 264 women) were randomised into the trial. In each treatment group, 64 men and 66 women were randomised. Of the 520 participants, 62 subjects (11.9 %) dropped out from the trial by the end of three treatment years, and for 458 subjects (88.1 %, 225 men, 233 women) the variable for atherosclerotic progression could be constructed.

Assessment of atherosclerotic progression

Equipment: Two identical Biosound Phase 2 systems were used (Biosound, Indianapolis, IN, USA) equipped with a 8-10 MHz annular array transducer, with a measurement precision of 0.03 mm.[27] The scannings were videotaped with PAL S-VHS Panasonic AG 7330E VCR.

Observers: Four ultrasound technicians (AM, JT, PV, RP) trained in arterial scanning several months to years prior to the study carried out the scannings. An experienced physician (Riitta Salonen) was the supervisor.

Scanning (imaging) procedure and videorecording: The ultrasonographic scanning of the common carotid arteries (CCA), the carotid bulbs and the proximal internal carotid artery (ICA) was performed after a supine rest of 10 minutes, the subject in the supine position. Both longitudinal and cross-sectional images were displayed. The scanning was started with a diagnostic examination of entire accessible carotid tree, to find the most severe

lesions. Secondly, the site of the greatest IMT at baseline in the CCA far wall was located and scanned thoroughly. This area was scanned from three angles: anterolateral, lateral and posterolateral.

Measurement from videotapes: All IMT measurements (both baseline and follow-up) from videotapes were made at the same site and angle at all examinations of each subject, which was the site with the greatest IMT (in any angle) which was clearly visible at baseline in the far wall of in CCA below the bulb. At this location IMT was measured in diastole for a length of 10 mm (or shorter, if not visible) in one angle for the far wall. Most often this was the distal centimetre of CCA. All IMT measurements were carried out after the 36-month examination by one very experienced technician (JT).

Ultrasound image analysis: Computer analysis of ultrasound images to measure IMT was performed with a reading station equipped with Data Translation DT 2861 video frame grabber interfaced to a Panasonic AG 7355 VCR. The Prosound software, developed by Robert Selzer, utilising automated boundary detection was used. IMT was determined as the average difference at on the average 100 points between intima/lumen and media/adventitia interface.[28]

Measurement Variability: Three technicians (AM, JT, PV) scanned 10 subjects twice at a weeks' interval in 1995. The videotapes from all scannings were read by one observer. The repeat correlations for the mean CCA-IMT were 0.988, 0.995 and 0.998 and pairwise inter-observer correlations 0.975, 0.983 and 0.995.

Construction of the main outcome variable: Atherosclerotic progression was defined a priori as the

linear regression slope of the mean common carotid IMT over six or seven points of follow-up time (0, 6, 12, 18, 24, 30 and 36 months). For 34 subjects, one follow-up was missing. First, the mean CCA-IMT from the right and the left side was averaged, and then the slope was computed across time-specific means.

Other measurements

Ascorbic acid was stabilised in heparin plasma with metaphosphoric acid immediately after plasma separation, and frozen at -80°C. Combined ascorbic acid and dehydroascorbic acid were determined with an HPLC method.[23] Heparin plasma for a-tocopherol was extracted with ethanol and hexane and measured by a reversed phase HPLC method.[25] Cholesterol and triglycerides were determined with enzymatic colorimetric methods.[14] Serum LDL cholesterol was measured based on precipitation using polyvinyl sulfate and HDL cholesterol after precipitation with magnesium chloride.[14] Plasma fibrinogen concentration was determined with a clotting method,[14] plasma homocysteine with an HPLC method,[30] and serum ferritin by an immunoradiometric assay (Bio Rad, Quantimune, Hercules, CA). Dietary intake of foods and nutrients was assessed at baseline by 4-day instructed food recording. Physical activity was assessed by 12-month checked questionaire.[29] Blood pressure was measured manually in sitting position after a rest of 10 minutes, three measurements at 3 minutes' intervals.

Statistical methods

All study participants for whom the main outcome variable was available, were included in the statistical analysis. Analyses were according to the intention-to-treat principle. As the subjects were randomised separately in

four strata (smoking men, non-smoking men, smoking women, non-smoking women), this stratification was maintained also in the statistical analysis. As the a priori power calculations were based on stratified analysis in men and women, the primary statistical analysis was done in these two strata (Tables 3 and 4).

To test the consistency of results, the outcome variable, the slope of the mean CCA-IMT over all available follow-up assessments, was used both as a continuous variable in general linear models and as a dichotomous variable in logistic models. The cut-off for the dichotomisation was the median among all 225 men. The use of gender-specific cut-off did not influence the results. Odds ratios were estimated as antilogarithms of coefficients and their confidence intervals (CI) based on normality assumption of SPSS 8.0 for Windows.

Three dummy variables were constructed to indicate whether the participant was randomised to receive only vitamin E, only vitamin C or both vitamins, and these were entered jointly in logistic models. The comparisons in the linear models were between each treatment group and all other groups.

As the distribution of the slope of mean CCA-IMT was not perfectly normally distributed, we used non-parametric methods to test the significance of the heterogeneity (Kruskal-Wallis variance analysis) of outcome between the four treatment groups and the difference between the groups randomised to both vitamins and others (Mann-Whitney test). In spite of one-sided hypotheses, p-values are reported as two-sided.

Results

Adverse events, compliance and adherence to treatment

Six study participants died during the first three study years. All of these were men. In the placebo group, there was one death due to cardiac arrhythmia. In the vitamin E group there were three deaths, of which one was accidental, one due to alcohol intoxication and one sudden coronary death. One man in the vitamin C group died of subarachnoid haemorrhage and one man in the double vitamin group due to complications of carotid endarterectomy.

The distribution of the 62 drop-outs according to the cause of drop-out and treatment group is presented in table 1 separately for men and women in the randomised groups. There were no differences between the randomised groups.

On the basis of count of returned tablets, during the whole trial on the average 94.9% of tablets were used, with almost no differences between either strata or treatment groups.

Baseline characteristics

The distributions of the main baseline characteristics of male and female study participants are shown in table 2. The smoking men had lower serum total, LDL and HDL cholesterol, plasma total ascorbate, a-tocopherol and b-carotene concentrations and greater both baseline mean IMT and increase of the mean IMT in three years (not shown) than the other groups. Both smoking men and smoking women had lower dietary vitamin C intake and higher dietary saturated fat intake and plasma fibrinogen

than the non-smokers. Of smoking men, 20.2% but of smoking women only 12.1% had plasma total ascorbate <25 mmol/L. Among both smokers and non-smokers, men had lower plasma total ascorbate, a-tocopherol and b-carotene levels and higher dietary intake of saturated fats, serum homocysteine levels and baseline CCA-IMT than women. Among men but not in women, smokers had a greater mean baseline CCA-IMT than non-smokers ($p<0.001$ for all differences). There were no significant differences between the randomised treatment groups within any stratum.

Change in plasma vitamin E and C levels

In men, the mean plasma a-tocopherol concentration increased in the placebo group from 31.0 to 33.2 mmol/L (by 7.2%), in the vitamin E group from 31.7 to 60.1 mmol/L (by 89.2%), in the vitamin C group from 32.3 to 33.9 mol/L (by 5.1%) and in the group randomised to both vitamins from 32.1 to 55.2 mmol/L (by 71.9%). The respective changes of plasma total ascobate concentration were -5.0, 3.8, 71.5 and 59.9%. In women, plasma a-tocopherol concentration increased in placebo, vitamin E, vitamin C and double vitamin groups by 5.6, 82.0, 4.0 and 75.4% and plasma total ascorbate by -1.1, 2.5, 47.1 and 46.1%, respectively ($p<0.001$ for heterogeneity for all comparisons).

Atherosclerotic progression

The average unadjusted increase (slope) of the mean CCA-IMT was 0.020 mm/year among men who were randomised to only placebo, 0.018 mm/year in those who received only vitamin E, 0.017 mm/year in men who received only vitamin C and 0.011 mm/year in those who received both vitamins ($p=0.043$ for heterogeneity). The IMT progression was

significantly less in men who were randomised to both vitamins, compared with all other men (p=0.009). The respective means in women were 0.016, 0.015, 0.017 and 0.016 mm/year (not significant).

Of all baseline measurements, serum ferritin and total cholesterol concentrations were most predictive of IMT progression in a step-up linear regression model in men. These and indicator variables for predictive baseline examination months were entered as covariates in linear covariance models predicting IMT progression (table 3). The covariate-adjusted IMT increase was 50.9% less (0.009 vs. 0.018 mm/year) in men who received both vitamin E and C, compared with other men (p=0.049). Differences between other supplementation groups were not statistically significant. None of treatment effects were significant in women.

In men, the proportion of those who experienced progression was reduced by 74% (95% CI 36-89%, p=0.003, table 4) in the group randomised to receive both vitamins, as compared with those who received only placebo. The respective treatment effects were non-significant in groups that received only vitamin E or vitamin C, although there were trends towards protection (table 4). These results were unaffected by the choice of covariates. In women, the probability of atherosclerotic progression was similar in all four randomised groups.

In smoking men, the preventive effect of vitamin E on atherosclerotic progression was larger than in non-smoking men (figure 1). In men who received only vitamin E there was 79% (95% CI 6-95%, p=0.04) less atherosclerotic progression and in those who received both vitamins, 93% (95% CI 63-99%, p=0.002) less atherosclerotic progression than in men who received only

placebo (table 5). In smoking men, there was a nonsignificant trend towards protection also among men who were randomised to only vitamin C. There were no statistically significant effects on the probability of atherosclerotic progression in either non-smoking men, smoking women or non-smoking women. Again, entering any additional covariates or deleting any of the entered covariates did not change these results qualitatively and had only very minor effect on the estimates of odds ratio.

Discussion

The present findings are the first demonstration in healthy persons of an atherosclerotic disease preventing effect of supplementation with antioxidative vitamins. Our study suggests that the benefit may be limited to men, and possibly to men who are at increased oxidative stress such as smokers or those who have insufficient status of dietary or endogenous antioxidants. The observed effect modification by gender and smoking status needs to be retested in further clincal trials.

As smoking men had considerably lower baseline levels of both plasma a-tocopherol and ascorbate, it is possible that the confinement of the observed benefit in this group could be simply due to the greater increase of these vitamins due to supplementation. The progression rate in smoking men who received vitamin E and C supplements was lower than in non-smoking men receiving placebo. Thus, in this study the preventive effect of the supplementation was at least equal to the atherosclerosis promoting effect of smoking. This is not a trivial effect from the public health point of view. On the basis of these findings, reasonable doses of vitamins E and C jointly can be recommended for regularly smoking men with

at least mild hypercholesterolemia. Recommendations concerning other kinds of persons can not be made on the basis of our current findings.

Both the vitamin E and C supplements were safe. There were neither excess deaths nor excess other adverse events in the groups randomised to supplements, although the sample size was not designed to detect effects on either deaths or other disease events. Both the adherence to treatment and the bioavailability of the supplements were good, judged based on increases of plasma vitamin levels. The drop-out rate during the trial was exceptionally low. The observed atherosclerotic progression in the placebo group was of the expected magnitude, suggesting that a potential "healthy participant effect" was small if any. However, the baseline vitamin E and C levels were higher than expected, especially vitamin C in women. This attenuated the achieved percentage increase in plasma vitamin levels and could be a partial explanation for the lack of effect on atherosclerotic progression in women. An alternative explanation is that women in general do not benefit from vitamin E or C supplements, as they have more effective endogenous antioxidative defence systems and in most Western cultures, more diversified diet than men.

In conclusion, this double-blind randomised clinical trial shows that a combined supplementation with reasonable doses of both vitamin E and vitamin C for at least three years can retard the progression of common carotid atherosclerosis substantially in regularly smoking men with at least mild hypercholesterolemia. This preventive effect may be generalizeable to all men. However, this study does not provide evidence for any substantial preventive effect in post-menopausal women, although a small benefit can not be ruled out. As common

carotid plaques and increased intima-media thickness have been shown to predict coronary events,[26] this observation may imply benefits with regard to other atherosclerosis-related events.

Contributors

JT Salonen and HE Poulsen were the principal investigators, wrote the protocol, and supervised the study. JT Salonen drafted the paper, K Nyyssönen, J Kaikkonen and E Porkkala-Sarataho supervised chemical analyses, R Salonen the ultrasound examinations and measurements, R Salonen, H-M Lakka, TA Lakka, T-P Tuomainen and V-P Valkonen performed clinical examinations and treatment, S Voutilainen, T Rissanen and U Ristonmaa the food recordings and gave dietary advise, L Leskinen planned time tables and co-ordinated subject visits, and JT Salonen, K Ronkainen, K Nyyssönen and S Voutilainen carried out data analyses.

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Cause for drop-out	Men					Women		
	Placebo	Vitamin E	Vitamin C	Both vitamins	Placebo	Vitamin E	Vitamin C	Both vitamins
Death	1	3	1	1	0	0	0	0
Severe adverse event	2	1	1	0	1	2	1	5
Adverse event	4	1	2	2	1	3	2	0
Refusal or other reason	5	3	1	1	6	3	3	6
Total	12	8	5	4	8	8	6	11

Table 1. **The causes for drop-outs in the four treatment groups for men and women.**

Baseline characteristic	Smoking men (n=100)			Non-smoking men (n=125)			Smoking women (n=110)			Non-smoking women (n=123)		
	Mean	Mini-mum	Maxi-mum	Mean	Minimum	Maxim-um	Mean	Minim-um	Maximum	Mean	Minimum	Maximum
Age (years)	59.5	46.0	70.0	60.4	45.4	70.0	58.1	47.1	69.6	60.9	46.8	70.4
Serum cholesterol (mmol/L)	6.05	3.41	8.32	6.53	4.39	9.92	6.22	4.42	8.86	6.73	4.42	11.57
LDL cholesterol (mmol/L)	4.33	1.42	6.36	4.73	2.45	8.14	4.25	2.57	6.91	4.67	2.03	9.05
HDL cholesterol (mmol/L)	1.12	0.55	1.83	1.14	0.68	2.21	1.35	0.69	2.75	1.43	0.68	2.55
Serum triglycerides (mmol/L)	1.55	0.38	4.40	1.73	0.51	7.51	1.47	0.54	4.59	1.63	0.45	21.60

Plasma fibrinogen (g/L)	3.79	2.1	5.5	3.47	2.1	5.4	3.83	2.4	5.6	3.59	2.2	5.4
Plasma total ascobate (mmol/L)	57.4	5.3	138.5	68.1	12.3	131.8	69.8	11.2	138.1	82.5	21.2	127.9
Plasma a-tocopherol (mmol/L)	29.7	14.7	48.0	33.5	19.4	60.7	31.2	19.2	52.8	35.4	19.9	54.3
Plasma b-carotene (mmol/L)	0.28	0.02	0.95	0.39	0.02	2.47	0.44	0.08	1.97	0.59	0.03	2.03
Plasma homocysteine (mmol/L)	10.8	5.9	25.1	10.5	6.1	19.2	9.5	4.5	16.9	9.3	4.7	16.3
Serum ferritin (mg/L)	120.0	12	376	142.3	9	1235	88.7	8	1090	66.7	5	414
Cigarettes/day	17.3	0	60	0.2	0	4	12.9	0	28	0.1	0	4
Intake of saturated fat (% of energy)	17.2	9.2	29.0	15.0	7.1	27.0	16.9	9.0	28.2	14.3	6.9	22.7

Dietary vitamin E (mg/1000 kcal/d)	5.0	2.2	11.3	5.3	2.5	12.4	5.1	2.2	9.1	5.5	2.8	9.3
Dietary vitamin C (mg/1000 kcal/d)	42.6	3.3	211	49.5	6.1	191	56.5	13.5	322	75.1	8.7	325
Alcohol intake (g/wk)	111	0	491	77	0	440	39	0	225	15	0	161
Total physical activity (min/wk)	193	15	600	215	20	655	205	30	640	232	20	905
Weight (kg)	77.2	51.4	103.1	79.6	53.2	96.3	66.6	45.4	90.0	66.8	46.5	91.9
Waist-to-hip circumference ratio	0.95	0.81	1.04	0.95	0.80	1.03	0.84	0.69	0.97	0.81	0.72	0.95
Systolic blood pressure (mmHg)	132.3	97.7	188.3	131.2	97.3	171.7	130.3	97.0	190.0	130.1	93.3	184.7
Diastolic blood pressure (mmHg)	78.8	55.7	99.3	81.4	60.7	99.3	76.2	51.3	99.3	78.5	58.7	99.3

Mean CCA-IMT (mm)	1.10	0.62	2.04	1.04	0.55	2.53	0.92	0.60	2.23	0.92	0.59	1.49

Table 2. Distributions of the main baseline characteristics of participants in the four randomization strata.

Supplement	Men (n=225)					Women (n=233)				
	Yes		No		P	Yes		No		P
	Mean (n)	SE	Mean (n)	SE		Mean (n)	SE	Mean (n)	SE	
Vitamin E (n=115)	0.0118 (56)	0.0050	0.0143 (169)	0.0022	0.571	0.0165 (59)	0.0046	0.0170 (174)	0.0021	0.904
Vitamin C (n=120)	0.0119 (59)	0.0050	0.0142 (166)	0.0022	0.600	0.0174 (61)	0.0046	0.0160 (172)	0.0021	0.732
Both vitamins (n=113)	0.0086 (58)	0.0050	0.0175 (167)	0.0022	0.049	0.0170 (55)	0.0047	0.0164 (178)	0.0020	0.895

Table 3. **The mean adjusted 3-year change* of the mean carotid artery intima-media thickness in participants who received vitamin E and C supplements in a multivariate general linear model.**

CI denotes confidence interval.

*Change estimated as the linear slope over 6-monthly assessments of mean IMT (mm/year).

† Statistical significance of contrasts to the double-placebo group.

Covariates in the model for both men and women are serum cholesterol and ferritin concentrations, and three indicator variables for baseline examination months.

Supplement	Men (n=225)			Women (n=233)		
	OR	95%CI	P	OR	95%CI	P
Vitamin E (n=115)	0.56	0.23, 1.36	0.200	1.05	0.48, 2.32	0.903
Vitamin C (n=120)	0.44	0.19, 1.06	0.066	1.08	0.49, 2.36	0.857
Both vitamins (n=113)	0.26	0.11, 0.64	0.003	1.36	0.60, 3.04	0.461

Table 4. **The effect of vitamin E and C supplements on the probability of atherosclerotic progression* in multivariate logistic models.**

* The slope of the mean IMT dichotomized at median (0.82 mm/year) for men.

OR denotes odds ratio and CI confidence interval. Three indicator variables for the three supplementation groups (double placebo as the reference group, n=106) were entered with age, serum cholesterol and ferritin concentrations, systolic blood pressure, and 11 indicator variables for baseline examination months.

Supplement	Smoking men (n=100)			Non-smoking men (n=125)			Smoking women (n=110)			Non-smoking women (n=123)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Vitamin E (n=115)	0.21	0.05, 0.94	0.041	1.07	0.31, 3.72	0.918	0.76	0.22, 2.62	0.666	1.13	0.36, 3.59	0.828
Vitamin C (n=120)	0.45	0.11, 1.82	0.260	0.30	0.08, 1.08	0.065	0.69	0.19, 2.53	0.574	0.99	0.32, 3.08	0.991
Both vitamins (n=113)	0.07	0.01, 0.37	0.002	0.55	0.17, 1.81	0.325	1.48	0.42, 5.13	0.540	1.18	0.34, 4.09	0.794

Table 5. **The effect of vitamin E and C supplements on the probability of atherosclerotic progression in multivariate logistic models.**

OR denotes odds ratio and CI confidence interval.

*Three indicator variables for the three supplementation groups (double placebo as the reference group, n=110) were enter d with age, serum cholesterol and ferritin concentrations, systolic blood pressure, and 11 indicator variables for baseline examination months.

Legend for figure:

Figure 1: Risk-factor-adjusted relative risk (odds ratio) of atherosclerotic progression in treatment groups and four randomisation strata. Three indicator variables for the three supplementation groups (double placebo as the reference group, n=110) were entered with age, serum cholesterol and ferritin concentrations, systolic blood pressure, and 11 indicator variables for baseline examination months.

Claims

1. A pharmaceutical delivery system for oral delivery of the antioxidants vitamin C and vitamin E to obtain high concentrations thereof and a controlled ratio between vitamin C and vitamin E in blood plasma in humans or animals, c h a r a c t e r i z e d in that it has a slow release of vitamin C and a plain release of vitamin E.

2. A pharmaceutical delivery system according to claim 1, c h a r a c t e r i z e d in that it is a system comprising a tablet comprising two or more different delivery principles , wherein

(A) one delivery principle comprises
  (i) vitamin C
  (ii) a pharmaceutically acceptable excipient for controlling the slow release of vitamin C
  (iii) other pharmaceutically acceptable excipients
(B) another delivery principle comprises
  (i) vitamin E
  (ii) pharmaceutically acceptable excipients.

3. A pharmaceutical delivery system according to claim 1 or 2, c h a r a c t e r i z e d in that the ratio between vitamin C and vitamin E in the blood plasma varies between 1:10 and 10:1, preferably 1:5 and 5:1, and especially 1:1 - 3:1.

4. A pharmaceutical delivery system according to any of the preceding claims, c h a r a c t e r i z e d in that the ratio between vitamin C and vitamin E in the blood plasma is about 2.2:1.

5. A pharmaceutical delivery system according to any of the preceeding claims, c h a r a c t e r i z e d in that the concentration of vitamin C in blood plasma is between 10 µmol/litre and 180 µmol/litre, preferably 20 µmol/litre and 180 µmol/litre, and the concentration of vitamin E is between 15 µmol/litre to 180 µmol/litre , preferably 50 µmol/litre and 180 µmol/litre.

6. A pharmaceutical delivery system according to any of the preceeding claims, c h a r a c t e r i z e d in that vitamin C is ascorbic acid and vitamin E is selected from the group comprising d-α-tocopheryl acetate, d-α-tocopheryl acid succinate, d-α-tocopherol, d-β-tocopherol, d-γ-tocopherol, d-δ-tocopherol, d-α-tocotrienol, d-β-tocotrienol, d-γ-tocotrienol, d-δ-tocotrienol, dl-α-tocopherol, dl-α-tocopheryl acetate, dl-α-tocopheryl calcium succinate, dl-α-tocopheryl nicotinate, dl-α-tocopheryl linoleate/oleate and all other possible derivatives or stereo isomeric forms of the above compounds.

7. Use of a combination of vitamin C and vitamin E for the preparation of a drug or drug system for treating or preventing atherosclerosis or other diseases or conditions responsive to antioxidants, wherein said vitamins are incorporated in the patients blood plasma in high concentrations and in a controlled ratio, c h a r a c t e r i z e d in that the drug has a slow release of vitamin C and a normal release of vitamin E.

8. Use according to claim 7, c h a r a c t e r i z e d in that the ratio between vitamin C and vitamin E in the blood plasma varies between 1:10 and 10:1, preferably 1:5 and 5:1, and especially 1:1 and 3:1.

9. Use according to claim 8, c h a r a c t e r i z e d in that the ratio between vitamin C and vitamin E in the blood plasma is about 2.2:1.

10. Use according to any of the claims 7 to 9, c h a-r a c t e r i z e d in that the concentration of vitamin C in blood plasma is between 5 µmol/litre and 80 µmol/litre, preferably 20 µmol/litre and 80 µmol/litre, and the concentration of vitamin E is between10 µmol/litre and 180 µmol/litre , preferably 50 µmol/litre and 180 µmol/litre.

A pharmaceutical delivery system for vitamin C and vitamin E and use of a combination of vitamin C and E for preventing or treating conditions involving oxidative stress

ABSTRACT

This invention relates to a pharmaceutical delivery system for obtaining a defined ratio in the blood plasma of vitamin C and E and high concentrations thereof. The pharmaceutical delivery system of the invention is useful for the prevention and progression of disease processes or for the treatment of pathological conditions relating to imbalance between oxidants and antioxidants in the blood plasma of humans or animals.